

In the Claims

The following list of pending claims replace any prior list of claims.

1. (Original) A method for the preparation of evolved microorganisms permitting a modification of metabolic pathways, comprising the following steps: a) preparing a modified microorganism by genetic modification of cells of an initial microorganism so as to inhibit the production or consumption of a metabolite when that microorganism is grown on a defined medium, thereby impairing the ability of that microorganism to grow; b) culturing the modified microorganism thereby obtained on said defined medium to cause it to evolve, where the defined medium can contain a co-substrate to allow such evolution; and c) selecting a modified microorganism able to grow on said defined medium, if necessary with a co-substrate.
2. (Original) The method as claimed in claim 1, wherein the metabolic pathway is selected from the group consisting of: biosynthesis pathways of amino acids, synthesis pathways of nucleic acids, synthesis pathways of lipids, and metabolism pathways of sugars.
3. (Original) The method as claimed in claim 2, wherein the modified metabolic pathway is a biosynthesis pathway of amino acids.
4. (Original) The method as claimed in claim 3, wherein the modified metabolic pathway is a biosynthesis pathway of an amino acid chosen from among: methionine, cysteine, threonine, lysine, or isoleucine.
5. (Original) The method as claimed in claim 2, wherein the modified metabolic pathway consumes NADPH.
6. (Original) The method as claimed in claim 1, wherein the modification made in step a) favors the reduction of NADP to NADPH.
7. (Original) The method as claimed in claim 2, wherein the modification made in step a) favors the reduction of NADP to NADPH.

8. (Original) The method as claimed in claim 1, wherein the evolved microorganism possesses at least one evolved gene coding for an evolved protein, the evolution of which replaces the inhibited metabolic pathway by a new metabolic pathway.

9. (Original) The method as claimed in claim 8, wherein it includes a step d) consisting of the isolation of the evolved gene coding for the evolved protein.

11. (Original) The method as claimed in claim 9, wherein the evolved gene is introduced, in an appropriate form, into a production microorganism intended for the production of the evolved protein.

12. (Original) The method as claimed in claim 1, wherein it includes an additional step a1) consisting of the introduction of at least one heterologous gene coding for a heterologous protein, which heterologous gene is to cause the evolution of a new metabolic pathway preparatory to step b) in which the modified microorganism is grown.

13. (Original) The method as claimed in claim 12, wherein it includes a step d) consisting of the isolation of the evolved gene coding for the evolved protein.

14. (Original) The method as claimed in claim 13, wherein the evolved gene is introduced, in an appropriate form, into a production microorganism intended for the production of the evolved protein.

15. (Withdrawn) An evolved microorganism obtainable by a method according to claim 1.

16. (Withdrawn) A method for the preparation of an evolved protein, wherein an evolved microorganism according to claim 15 is grown in an appropriate culture medium for the production of the evolved protein.

17. (Withdrawn) A method as claimed in claim 16, wherein the evolved protein produced is purified.

18. (Withdrawn) An evolved gene coding for an evolved protein obtainable by a method according to claim 9.

19. (Withdrawn) An evolved gene coding for an evolved protein obtainable by a method according to claim 13.

20. (Withdrawn) An evolved protein obtainable by a method according to claim 16.

21. (Withdrawn) An evolved protein obtainable by a method according to claim 17.